

Importantly, the deposited seed of inbred maize line NP2171 meets the written description requirement of the claimed invention as recognized in *Enzo Biochem v. Gen-Probe Incorporated*, 296 F.3d 1316, 1325 (Fed. Cir. 2002): “In light of the history of biological deposits for patent purposes, the goals of the patent law, and the practical difficulties of describing unique biological materials in a written description, we hold that reference in the specification to a deposit in a public depository, which makes its contents accessible to the public when it is not otherwise available in written form, constitutes an adequate description of the deposited material sufficient to comply with the written description requirement of § 112, P 1.” Thus, since the deposited line NP2171 is part of the written description of the invention, **it can be used to apprise one skilled in the art as to the subject matter of the claims.** By analyzing the breeding history records of any particular maize plant material and/or through genetic and phenotypic testing of this plant material and comparing this data to the genotypic and phenotypic data generated from growing the deposited maize inbred line NP2171 (which constitutes part of the specification), one skilled in the art can determine whether a particular plant is derived from inbred line NP2171, and thus can determine the probability that two particular traits were derived from NP2171. Applicant submits that one skilled in the art can determine, using the breeding history of a plant material, common knowledge and techniques that exist in the art at the filing date of the present application, and the teachings of the claim and specification (including the deposited maize inbred line NP2171) of the subject application, whether a particular plant material constitutes infringement of amended claim 42. With regard, to definiteness, no more is required. Therefore, Applicant respectfully submits that the indefiniteness rejection of amended claim 42 is not applicable and requests that it be withdrawn.

On page 3 of the Office Action, the Examiner states that neither individual traits nor their degree of expression is unique to NP2171. First, Examiner provides no basis for this statement. Second, uniqueness is not relevant to an indefiniteness rejection, and as such will not be addressed here.

The Examiner rejected claim 6 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the Examiner states “it is unclear how a male sterile plant would result from the non-male sterile maize plant of claim 2.” First, claim 2 does not claim a sterile plant. Second, the production of male sterile plants is a standard practice in

the art and is the basic procedure used in the industry to produce hybrid maize seed. The production of male sterile plants is described on pages 2 and 3 of the specification. For example, the specification discussed the common technique of manual or mechanical detasseling. Furthermore, page 2, lines 9-18 discuss using cytoplasmic male-sterile inbreds. CMS plants are fertilized with the pollen from another inbred that is not male-sterile. Several other methods for conferring genetic male sterility are discussed on page 2, lines 19-31 and page 3, lines 1-5. In view of what is commonly known and practiced in the breeding arts, Applicant respectfully submits that making the plant of claim 2 male sterile is clearly described and definite in the art and requests that the rejection be withdrawn.

Claims 7-8, 38, 40-41, and 42-46 stand rejected as indefinite. The Examiner states that the metes and bounds of a "single gene transferred traits," "single gene trait," or "transgene" are unclear, as the phrase or term does not carry with it any limitations as to the structural or physiological properties of the gene. Applicant has amended claim 7 to recite a "gene transferred trait," thereby eliminating reference to an unknown multiple of gene transferred traits. Applicant has also amended claims 9 and 11 to depend from claim 8, claim 12 to depend from claim 11, and 38 to cover a method of claim 37, further comprising a transgene.

The claims recite incorporating or transforming a gene into the claimed inbred line NP2171. The specification includes a discussion with citations to publications that describe transformation and breeding technologies. See pages 19-23 of the specification. The specification also describes the germplasm into which a gene is to be incorporated or transformed. Furthermore, the specification also describes and sets forth numerous genes that have been transformed into plants and subsequently bred into elite lines. Any one skilled in the art would understand what a gene is, how to transform maize plants with a gene, and how to introgress a gene by backcrossing and other breeding techniques into different genetic backgrounds. 35 U.S.C. 112, second paragraph, requires no more from a claim than making it clear to one skilled in the art what the claim encompasses. *PPG Industries, Inc. v. Guardian Industries Corp.* 75 F.3d 1558 (Fed. Cir. 1996). There is no lack of clarity in these claims as to what is meant, and what is being encompassed by these claims. The Examiner can easily determine the scope of the claims and determine their patentability based on 35 U.S.C. 102 and 103. No more is required. Applicant respectfully requests, in view of the amended claims and the above remarks, that the rejection of claims 7-8, 38, 40-41, and 42-46 under 35 U.S.C. 112, second paragraph, be withdrawn.

Claim 45 has been canceled.

Claim 48 has been amended as suggested by the Examiner.

Claim 6-15, 23-24, 29-49 stand rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such as way as to enable one skilled in the art to make and/or use the invention.

The Examiner states that Applicant has not disclosed or provided guidance for a:

- transformed or non-transformed maize inbred line NP2171 or parts thereof further comprising introgressed one or more single gene traits, or a trait such as herbicide tolerance, disease or insect resistance transferred by introgression such as backcrossing.
- obtention of F1 generation of subsequent generalization maize plants in addition to the single gene traits introgressed to the inbred or a method for producing said plants.

The Examiner further states that while transformation techniques are well within the level of one skilled in the art, the art is unpredictable and that undue experimentation would be required to express the phenotypes in different genetic backgrounds.

Applicant respectfully disagrees. As stated by the Examiner, the subject specification, combined with the common knowledge and practice by those persons skilled in the art, enables the transformation of a single transgene into plant material. The Examiner, however, states that introgression of a single gene into different genetic backgrounds is not enabled. This is not true. Introgression of a single transgene trait into elite germplasm from source material has been a standard practice in the art for many years. Attached to this Reply are several publications that describe standard plant breeding methods that are used to introgress a transgene into different genetic backgrounds.

Principles of Plant Breeding, 2<sup>nd</sup> Ed., Allard, A. (John Wiley & Sons, 1999) discusses in detail the use of backcrossing to introgress one or only a few specific characteristics into a superior variety:

The generally well adapted parent (the recurrent parent) into which an allele is to be substituted is involved in each backcross; the other parent (the donor parent) is involved in only the original cross. At the end of the backcrossing the allele (or alleles) being substituted will be heterozygous. Selfing after the last backcross produces a homozygosity for the allele (or alleles) being substituted and, coupled with selection, will result in a variety (or stock) with exactly, or very nearly exactly, the adaptedness, yielding ability, and quality characteristics of the recurrent parent but superior to that parent in respect to the particular characteristic(s) for which the improvement program was undertaken. Pg. 188

Also attached is a print out of University of Nebraska-Lincoln websites entitled *Overview of the Process of Plant Genetic Engineering and Backcross Breeding*. The *Overview* article briefly describes backcrossing of a transgene into an elite breeding line. In the *Backcross Breeding* article, the author discusses yield drag and yield lag. However, as the article discusses, these effects are not barriers to successfully introgressing a transgene into an elite variety. In other words, introgressing or transforming a gene into different backgrounds is not “inherently unpredictable” so as not to be enabled as asserted by the Examiner.

The Examiner refers to Hunsperger, stating that this publication discloses a single gene trait in the genetic background of introgressed into the genetic background of another, that didn't result in the expected gene trait. Applicant respectfully points out that the Hunsperger publication is directed to work in Petunias, not corn. In addition, introgression of the gene was successful in the majority of genetic backgrounds. The fact that they were unable to obtain success in one experiment does not mean that it could not be accomplished with a second experiment. **Furthermore, a claim cannot be rejected for lack of enablement “even though it listed elements that could form thousands of end products, some of which may not be operative. A claim may be invalid if the number of inoperative combinations becomes significant, forcing a person skilled in the art to experiment unduly to practice the claimed invention.”** *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.* 750 F.2d 1569 (Fed. Cir. 1984). Applicant respectfully submits that introgressing a transgene trait or transforming a transgene into different genetic backgrounds is well within the skill of persons skilled in the art, although it may require several generations of backcrossing and continual and careful selection of the desired phenotype and although a few transformations may be inoperative. Applicant submits that the specification enables one skilled in the art to introgress or transform a gene into different genetic backgrounds, including into F1 and subsequent generations.

The Examiner also recites the Kraft et al. article. The article is not really on point, because the purpose of backcross breeding is to break the linkage between closely linked genes by random recombination. After a number of backcross generations, the breakup of linkage disequilibrium will occur. It is also important to point out that even if phenotypic variations occur because of linkage disequilibrium, the end plant product can still contain the selected single transgene expressing its

gene product. **The fact that the phenotype of the host plant is slightly changed does not mean the process of transgene transformation or introgression is not enabled.**

Finally, the Examiner refers to Eshed et al. and epistatic interactions. Applicant points out that in the Eshed et al. article the experiments are based on introgressing large chromosome segments, quantitative trait loci, into otherwise homogenous backgrounds. See Fig. 4, which illustrates the large chromosome segments introgressed into nearly isogenic lines. The article simply does not apply to introgressing a transgene from one genetic background into another. Applicant also points out that in the first paragraph of the Discussion section, the author states “QTL mapping studies in conventional segregation populations ... have generally uncovered little evidence for epistasis.” Also, on page 1813, first column, the author states, “the genetic constitution of the lines can be easily manipulated to generate shorter introgressions that retain the phenotypic effects.” Applicant respectfully submits that the Eshed article, Hunsperger et al, nor Kraft et al. support the proposition that the introgression of a single transgene trait from one genetic background to another, or the transformation of a plant with a transgene, is not common in the maize breeding and biotechnological arts. Applicant respectfully requests that the 35 U.S.C. 112, first paragraph, rejection of claims 6-15, 23-24, 29-49 be withdrawn.

Claim 42 remains rejected and claims 6-15, 23-24, and 29-49 stand rejected under 35.U.S.C. 112, first paragraph, as containing subject matter that was not sufficiently described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Examiner states that the Applicant only describes the inbred line NP2171, deposit Accession No. PTA-2886, which has specific genotypic and phenotypic characteristics, wherein plants derived therefrom (F1 hybrids or subsequent generation plants) are not “disclosed or completely characterized,” either by genotype or phenotype. The Examiner further states that Applicant has not described any phenotypic trait that distinguishes NP2171 derived maize plants or introgressed NP2171 maize plants comprising linked quantitative or single genes of unknown characteristics, including F1 and subsequent generations, nor has Applicant described one or more transgenes or their phenotypic effects in particular maize backgrounds. The Examiner concludes that absent such a description, one skilled in the art would not know that Applicant was in possession of the plant of claim 42 and of claims 6-15, 23, 24, 29-49. Applicant continues to respectfully disagree.

First, with regard to NP2171 derived maize plants having a transgene as set forth in the claims, Applicant submits that transformation of a transgene into maize plants is supported by the specification pages 21-28, which describe a number of transgenic traits that are capable of being introduced into maize using techniques that were known to those skilled in the art at the filing date of this application. The specification also refers to a number of articles and patents describing in detail the maize transformation and breeding technologies. The skill and knowledge in the art was high at the filing date of the subject application, as illustrated in the specification. Clearly, transformation techniques and traits capable of being transformed, or introgressed, into maize germplasm was a matter of public knowledge and skill at the filing date of the subject application. The Guidelines for Examination of Patent Applications under 35 U.S.C. 112, ¶1, Written Description Requirement, Section II. Methodology for Determining Adequacy of Written Description states:

Patents and printed publications in the art should be relied upon to determine whether an art is mature and what level of knowledge and skill is in the art. In most technologies which are mature, and wherein the knowledge and level of skill in the art is high, a written description question should not be raised for original claims even if the specification discloses only a method of making the invention and the function of the invention.

Also, see *In re Hayes Microcomputer Products, Inc.* 25 USPQ2d 1241 , 1246 (Fed. Cir. 1992), where the Court stated:

Disclosing a microprocessor capable of performing certain functions is sufficient to satisfy the requirement of section 112, first paragraph, when one skilled in the art would understand what is intended and know how to carry it out.

Many aspects of the biotechnological arts are not as unpredictable as they once were. Thus, as with standard computer software programs, it should not be necessary to describe now common aspects of the biotechnological arts. Applicant respectfully submits that the terms **transgenes** and **introgressed genes** are sufficiently descriptive (sections of DNA) in and of themselves, in the context of the present invention, and that a person skilled in the art would understand that the claimed inbred maize line NP2171 is described and novel, and that person would conclude that the Applicant had possession of a NP2171 having a gene introgressed or transgene incorporated therein. Applicant also disagrees with the Examiner's position stated on page 7 of the Office Action, that the

NP2171 derived progeny must be “completely characterized” by genotype or phenotype. The standard for descriptiveness simply requires that the applicant describe the claimed invention such that one skilled in the art understands that the inventor had possession of the invention. The Examiner is setting a much higher standard than that which is required by law.

Claim 42 and claims 23-24, 29-30, 35-39, and 47-49 stand rejected under first paragraph of 35 U.S.C. § 112, which recites that “the specification shall contain a written description of the invention.” As already stated, the “written description requirement” of § 112 requires that the patent describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed subject matter. Vas-Cath, Inc. v. Mahukar, 935 F.2d 1555, 1563 (Fed. Cir. 1991); Regents of the Univ. Cal. v. Eli Lilly & Co., 119 F.3d 1599, 1566 (Fed. Cir. 1997). The purpose of the written description requirement is to prevent an inventor from overreaching by claiming an invention more broadly than that which the inventor can demonstrate he invented. An inventor shows possession of the claimed invention with words, structures, figures, diagrams or formulas that fully set forth the claimed invention. Lockwood v. American Airlines, Inc., 107 F.3d 1563, 1572 (Fed. Cir. 1997). As stated above, a deposit of material in public depository is considered in evaluating the written description requirement. Enzo, 296 F.3d at 1325.

Claims 1 and 2, which claim the inbred maize line NP2171, ATCC Accession No. PTA-2886, are described by the deposited seed and the description of the NP2171 in the specification. Claim 42 and claims 23-24, 29-30, 35-39, and 47-49 themselves, which were part of the patent application as-filed, describe the invention and would allow one of ordinary skill in the art to recognize that the inventor was in possession of what is claimed. Union Oil Co. of Cal. V. Atlantic Richfield Co., 208 F.3d 989, 998, n.4 (Fed. Cir. 2000) (an originally filed claim can satisfy the written description requirement). The PTO’s written description guidelines recognize a strong presumption of adequate written description of a claim present when the application is filed. See “Guidelines for Examination of Patent Applications Under the 35 U.S.C. § 112, ¶ 1 ‘Written Description’ Requirement,” 66 Fed. Reg. 1099, 1105 (Jan. 5, 2001).

The claims to plants derived from inbred maize line NP2171, including hybrids and subsequent generations having inbred line NP2171 as an ancestor, are directed to plants having a combination of NP2171 traits. These claims provide those skilled in the art with more than enough information to be able to distinguish the claimed plants from all other plants, because

current methods and techniques, using seed of the deposited line NP2171, can differentiate between the claimed plant and all other plants. As long as the claims provide enough information that one skilled in the art can distinguish the invention from the prior art, no more is required under 35 U.S.C. 112, first paragraph. Applicant respectfully submits that the claims adequately describe and distinguish the claimed invention from the prior art and demonstrate that Applicant had possession of the subject matter of claim 42 and claims 6-15, 23, 24, and 29-49.

Under certain circumstances, the original claim language itself may not provide an adequate written description or demonstrate that the inventor was in possession of the invention. See Written Description Guidelines, 66 Fed. Reg. at 1104. Since the claims at issue cover a number of generations of maize plants, the issue is raised by the Examiner as to whether the inventor was in possession of the entire scope of these claims. Furthermore, the Examiner states that the claimed invention set forth in the subject claims are not supported by an adequate written description, because the plants that are produced by the claimed process are not “completely characterized” by structure or phenotypic characteristics.

The amount of detail necessary to demonstrate possession of the invention varies depending on the predictability of the technology and the level of skill in the art. One skilled in the art, reading the original disclosure, must immediately discern the limitations at issue. Purdue Pharma L.P. v. Faulding Inc., 230 F.3d 1320, 1323 (Fed. Cir. 2000)(citing Waldemar Link GmbH & Co. v. Osteonics Corp., 32 F.3d 556, 558 (Fed. Cir. 1994)). In a predictable technology, where one of ordinary skill in the art can predictably practice invention with the disclosed method of making the invention, a less detailed description of the invention may be adequate. Written Description Guidelines, 66 Fed. Reg. at 1106.

Referring again to *In re Hayes*, the inventor in that case was not required to describe the internal structure of processor programmed with a software timer. The Court found that one of ordinary skill in the art would know how to program a microprocessor to perform the necessary function described in the specification, and therefore the inventor was not required to disclose the details of the processor. The Court stated that disclosing a microprocessor capable of performing certain functions is sufficient to satisfy the written description requirement when one skilled in the art would know what was intended and how to carry it out. It is important to note that persons skilled in the art would program the microprocessor differently, as it is recognized that there are a huge variety of ways to program a microprocessor(s) to get to the claimed



product or process. The Court clearly states that describing in general terms how a microprocessor would function, without detailing the actual programming to accomplish that function, meets the written description requirement, because one skilled in the art, based on current knowledge in the field, would know how to obtain the claimed invention.

Here, a maize breeder having ordinary skill in the art would be able to predictably obtain unlimited generations of maize plants that have as an ancestor NP2171 and that are capable of expressing NP2171 traits using conventional maize breeding techniques. The fact that such an NP2171 derived plant contains traits (i.e., details of the computer program) not described in detail does not mean that the claimed subject matter as a whole is not described. In other words, it is not necessary for the Applicant, in this case, to fully characterize, by genotype or phenotype, the invention.

Applicant also respectfully submits that all non-NP2171 traits are “conventional” features of the claimed invention, i.e., of NP2171-derived plants. It doesn’t matter what non- NP2171 traits are contained in the NP2171 plants, because the novel features are identified and described by the specification and the deposited maize inbred line NP2171. See, Synopsis of Application of Written Description Guidelines, Pages 1 and 7 of section entitled Written Description, Original claims, Decision, where “conventional features” are recognized as not essential to the claimed inventions. Applicant respectfully submits that one of ordinary skill in the art would immediately discern the novelty of the subject claims after reviewing the patent application as filed, which includes the deposited seed of NP2171, and recognize that the inventors had possession of the claimed invention.

Finally, the Examiner recites *University of California v. Eli Lilly* 119 F.3d 1567 (Fed. Cir. 1997) to support the 35 U.S.C. 112, first paragraph rejection. It is important to point out that the *Eli Lilly* case specifically states that all that is required under 35 U.S.C. 112, first paragraph, is a description that distinguishes the claimed invention from other materials. Again, Applicant does not believe that *Eli Lilly* presents an analogous set of facts. In that case, the invention was a DNA sequence that was not described in the specification. In this case, the technology is different in that the inbred maize line NP2171 is fully described by the deposited seed. Furthermore, as stated above, any one skilled in the art by using current methods and technologies, can distinguish progeny of NP2171 having at least two derived traits from other plants. Since Applicant’s claimed plants can be distinguished from other plants, Applicant

respectfully submits, that claims 42 and 6-15, 23-24, and 29-49 are adequately described, and requests that the 112, first paragraph, rejection of these claims be withdrawn.

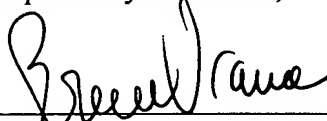
Claim 42 remains rejected and new claim 49 is rejected under 35 U.S.C. 102(b) or 103(a) in view of Mies et al. (U.S. Patent No. 5,792,906). Applicant respectfully disagrees for the following reason. Claims 42 and 49 are directed to a maize line derived from inbred maize line NP2171 of claims 1 or 2. It is well known that although one could try to develop the maize line NP2171 of the invention or one substantially similar, due to genetic variation amongst the different breeding partners and the recombination events that occur during breeding, it would be nearly impossible to do so. Indeed, the Examiner has confirmed that none of the cited references anticipate or make obvious the inbred maize line NP2171 of claims 1 and 2. Therefore, since the NP2171 is not anticipated or made obvious by the prior art, then it is impossible that the prior art anticipates or makes obvious plants made or derived from NP2171, because if the parent line is not capable of being produced via the teaching of the prior art, then one skilled in the art certainly cannot produce progeny that are derived therefrom. Furthermore, if the parent line is deemed non-obvious in view of the prior art, then a claim that is directed to only NP2171 derived progeny must also be non-obvious – as NP2171 is essentially inherent in all progeny. Simply stated, if NP2171 is unique, so must be its progeny. For these reasons, Applicant respectfully requests that the Examiner withdraw its 102(b) and 103(a) rejections of claims 42 and 49 in view of Mies et al.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned “Version With Markings To Show Changes Made.”

In view of the above amendments and remarks, it is submitted that the application is ready for allowance. If any additional information is needed, the Examiner is invited to call the undersigned attorney at (919) 541-8614.

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Respectfully submitted,

  
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Date: February 10, 2003

**Version With Markings To Show Changes Made**

**In the claims:**

Claim 45 has been cancelled.

The following claims have been amended:

5. (Amended) A maize plant, or parts thereof, having all the physiological and morphological characteristics of [a] the plant according to claim 2.
7. (Twice Amended) The maize plant, or parts thereof, according to claim 2, further comprising [one or more single] a gene transferred trait[s].
8. (Twice Amended) The maize plant, or parts thereof, according to claim 2 [7, wherein the plant or parts thereof have been transformed so that its genetic material contains one or more] further comprising a transgene[s] operably linked to one or more regulatory elements.
9. (Twice Amended) The maize plant according to claim [7] 8, wherein said [single] transgene [transferred trait comprises a gene] confers[ring] upon said maize plant tolerance to a herbicide.
11. (Twice Amended) The maize plant according to claim [7] 8, wherein said [single] transgene [transferred trait comprises a gene] confers[ring] upon said maize plant insect resistance, disease resistance or virus resistance.
12. (Twice Amended) A maize plant according to claim 11, wherein said transgene conferring upon said maize plant insect resistance is a Bacillus Thuringiensis Cry1Ab gene.
38. (Twice Amended) The method according to claim 37, wherein said one parent is the plant of inbred maize line NP2171, further comprising a [single] transgene [transferred trait].
40. (Twice Amended) A method comprising introgressing a [single] gene [trait] into inbred maize line NP2171, seed of said line having been deposited under ATCC Accession No. PTA-2886, using one or more markers for marker assisted selection among maize lines to be used in a maize breeding program, the markers being associated with said [a single] gene [trait], wherein the resulting maize line is inbred maize line NP2171 further comprising said [single] gene [transferred trait].
41. (Twice Amended) The method according to claim 40, wherein said [a single] gene [trait] comprises a Cry1Ab gene.
42. (Twice Amended) A NP2174-derived maize plant, or parts thereof, wherein at least one ancestor of said maize plant is the maize plant of claim 2, said maize plant expressing a

combination of at least two NP2174 traits selected from the group consisting of: a relative maturity of approximately 85 to 105 days based on the Comparative Relative Maturity Rating System for harvest moisture of grain, [acceptable to good grain quality, good] Eyespot resistance, [good] Common Rust resistance, [average] First Brood Corn Borer resistance, Gray Leaf Blight resistance, anther color of 17 (Munsell Code), silk color 3 days after emergence of 26 (Munsell code), husk tightness greater than that of A619, slightly curved row alignment compared to the straight row alignment of A619, greater ear height to base of top ear node compared to A619, [good Second Brood Corn Borer resistance, average early growth, good seedling vigor, early pollen shed, reliable late season plant health, average pollen shed, improved stalk strength and resistance to stalk diseases, acceptable late season intactness,] and adapted to the Northern Cornbelt regions of the United States.

43. (Amended) The maize plant, or parts thereof, of claim 5, wherein the plants or parts thereof have been transformed so that its genetic material contains [one or more] a transgene[s] operably linked to one or more regulatory elements.

46. (Amended) A method for producing a maize plant that contains in its genetic material [one or more] a transgene[s], comprising crossing the maize plant of claim 45 with either a second plant of another maize line, or a non-transformed maize plant of the line NP2171, so that the genetic material of the progeny that result from the cross contains the transgene[s] operably linked to a regulatory element.

48) (Amended) [A maize plant breeding program] The method of claim 47, wherein the plant breeding techniques are selected from the group consisting of[:] recurrent selection, backcrossing, pedigree breeding, restriction fragment length polymorphism enhanced selection, genetic marker enhanced selection, and transformation.

# **Principles of Plant Breeding**

**Second Edition**

ROBERT W. ALLARD



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phoretically detectable allelic combinations that had been present in the parents and in the generation  $F_1$  hybrid were again found in generations  $F_8$  through  $F_{12}$ . Nevertheless, yields changed little from generation  $F_9$  to  $F_{13}$ , and the frequency of clearly inferior plants, especially tall late plants subject to lodging, remained high during that five-generation interval. Population changes were much slower than had been the case in the earlier study, in which competition had been among only four well-adapted varieties, Atlas, Club Mariout, Hero, and Vaughn. This suggested that the problems that developed during generations  $F_9$  to  $F_{13}$  might have been that most of the competition in those generations was with plants that were clearly inferior agriculturally; if this was so, the removal of such plants from the population might more closely duplicate the sorts of competition that had prevailed in the earlier encouraging experiment with Atlas, Vaughn, Hero, and Club Mariout. Accordingly, the experiment was continued from generations  $F_{14}$  through  $F_{20}$ , during which period rigorous selection was practiced against obviously inferior types in generations  $F_{14}$ ,  $F_{15}$ , and  $F_{16}$ . Thereafter the survivors, mostly desirable agriculturally, were allowed to compete in generations 17, 18, 19, and 20, during which yields increased steadily, ultimately reaching a population mean slightly higher than the original  $F_1$  yield (118%) of the three parents. It is not known how much of this yield increment was due to favorable interactions at the phenotypic level among the components of the population; clearly, not all was due to this factor, because among the pure lines that were isolated from the mixture, some were equal in yielding ability to the mixed population. The three experiments described in this chapter thus suggest that segregation and recombination, aided by natural selection, sometimes improve yielding ability either by (1) repairing defects in segregants from already well-worked gene pools or (2) increasing the frequencies of pure lines that interact favorably with each other at the phenotypic level to give higher yields in mixture than in pure stand.

### BACKCROSS BREEDING

The backcross method of breeding has been widely used to improve homozygous varieties and breeding stocks, as well as inbred lines of outcrossing species that are generally superior in overall worth but deficient in one or only a few specific characteristics. As the name implies, the method calls for a series of backcrosses to a generally superior variety or stock, during which some particular character to be improved is maintained by selection. Beginning in the  $F_1$  generation and continuing for several generations, hybrid plants with the allele (or alleles) to be substituted are successively backcrossed to the parent to be improved. Thus, the generally well-adapted parent (the recurrent parent) into which an allele is to be substituted is involved in each backcross; the other parent (the donor parent) is involved in only the original cross. At the end of the backcrossing the allele (or alleles) being substituted will be heterozygous. Selfing after the last backcross produces homozygosity for the allele (or alleles) being substituted and, coupled with selection, will result in a variety (or stock) with exactly, or very nearly exactly, the adaptedness, yielding ability, and quality characteristics of the recurrent parent but superior to that parent in respect to the particular characteristic(s) for which the improvement

program was undertaken. It is apparent that this method of breeding, in contrast with virtually all other methods, provides plant breeders with a unique degree of control of allelic frequencies in their populations.

Although line breeding, which features repeated crossing in a line derived from a common ancestor, has been used by animal breeders for centuries in attempts to fix breed characteristics in domestic animals, the great potential of backcross breeding in plant breeding seems to have escaped attention until Harlan and Pope (1922) pointed out the possibilities of the method in improving self-pollinated small grains. They observed that the backcross method had been "largely if not entirely neglected in definite programs to produce progeny of specific types." They suggested the probability that there were many instances in which backcrossing would be of greater value than the more common procedure of selecting during a program of selfing following hybridization. It was also in 1922 that Briggs (1938) started an extensive and long-continued program to add resistance to various diseases to well-established varieties of self-pollinated cereals. Briggs emphasized that "within certain limits the method was scientifically exact because the morphological and agricultural features of the improved variety could be described accurately in advance, and because the same variety could, if desired, be bred a second time by retracing the same steps."

#### *The Genetic Basis of Backcross Breeding*

The  $F_2$  generation obtained by selfing the  $F_1$  of the cross  $AA \times aa$  is expected to be made up of  $1/4 AA:1/2 Aa:1/4 aa$  individuals. Even though half of the individuals are homozygotes, only  $1/2$  of these homozygotes—or  $1/4 F_2$  of the total population—will be of the superior and desired genotype of the recurrent parent, say  $AA$ . However, if instead of selfing the  $F_1$ , it is backcrossed to the recurrent parent, the expectation in the first backcross is  $1/2 AA$  and  $1/2 Aa$  individuals. Thus, in the backcross, half of the progeny are expected to be desired  $AA$  homozygotes. The same expectation holds for each gene pair by which the two parents differ. If additional backcrosses are made to the recurrent parent, the hybrid population progressively becomes more like that parent instead of breaking up into  $2^N$  homozygous genotypes as with selfing. If, for example, the parents differ by 10 gene pairs and no selection is practiced, six backcrosses will produce a population in which 99.4% of individuals are expected to be homozygous and identical with the recurrent parent at all 10 loci; that is, virtually all alleles from the donor parent will have been eliminated after six backcrosses. The rate at which alleles that enter a hybrid from the donor parent are eliminated during backcrossing will, of course, be affected by linkage. Suppose, in a backcrossing program undertaken to transfer the desirable allele  $A$  from a generally undesirable parent (the donor parent) to a generally superior variety (the recurrent parent), that an undesirable allele  $b$  is linked to allele  $A$  of the donor parent. The genotype of the  $F_1$  hybrid will be  $Ab/aB$  and selection for allele  $A$  in the first backcross generation will tend to pull along allele  $b$ , thus slowing the process of obtaining the desired  $AB$  recombinant genotype. However, because allele  $B$  is reintroduced with each backcross, there will be recurring opportunities for crossovers to occur. Assuming that selection is practiced only for  $A$  in



all generations, the allele being transferred, the probability of eliminating allele *b* will be  $1 - (1 - c)^N$ , in which *c* is the crossover rate between *A* and *b* and *n* is the number of backcrosses to the recurrent parent. Hence if allele *B* is located 50 or more crossover units from *A*, the probability that allele *b* will be eliminated during six backcrosses is  $1 - (0.5)^6 = 0.984$ . In a selfing series with selection only for allele *A*, the corresponding probability is 0.50. These probabilities become progressively smaller, of course, with closer linkage between alleles *A* and *b*; for example, if the recombination fraction is 0.01, the probability is  $1 - (0.99)^6 = 0.06$ , indicating that the unfortunate linkage would probably have been broken in about 6 among 100 backcross lines, in contrast to only about 2 among 100 lines derived by selfing. The probability of obtaining a desirable recombinant is clearly greater with backcross breeding than with selfing, assuming, as is often the case, that selection cannot be practiced against undesirable alleles that are linked to desirable alleles. However, should it be possible to practice effective selection against undesirable alleles that are linked to one or more desirable alleles under transfer, both selfing and backcrossing afford opportunities for obtaining desired recombinants. In fact, a selfing series may, under such circumstances, be more efficient than backcrossing because crossing over is possible in both male and female gametogenesis, whereas with backcrossing, effective crossing over cannot take place in the recurrent parent. Effective selection is, however, usually not possible with alleles affecting characters of low heritability. Hence, in dealing with characters of low heritability, the backcross procedure is nearly always advantageous because it deals with all undesirable alleles automatically, regardless of heritability.

It is clear that recurrent backcrossing, even in the absence of selection, is a powerful tool for achieving homozygosity and that any population derived by backcrossing must rapidly converge on the genotype of the recurrent parent. When recurrent backcrossing is made the driving force in a plant-breeding program, the genotype of the recurrent parent will be modified only in respect to the allele or alleles being transferred, which must, of course, be maintained by selection. Properly executed, backcross breeding programs thus allow all of the desirable characteristics of the recurrent parent to be recovered and retained, except for the possibility that alleles very tightly linked to the allele or alleles being transferred will be modified inadvertently. This is both the strength and weakness of the method. Recurrent backcrossing provides a precise and certain way of making gains of predictable value, with little possibility that uncontrolled segregation will produce unanticipated weaknesses that may be difficult to ascertain in necessarily finite periods of testing and evaluation of a novel backcross-derived variety. At the same time, the method has a disadvantage: it places an upper limit on the amount of advance, one that may be lower than that possible when segregation is less rigidly controlled.

#### *Selecting the Recurrent Parent*

Because a series of backcrosses reproduces the genotype of the recurrent parent precisely, except for the character under transfer, it is obvious that backcross programs must be based on suitable recurrent parents. In well-established crops

successful varieties, successful inbred lines and  $F_1$  hybrid varieties grown in any area almost certainly represent the end products of long periods of Darwinian evolution in which natural selection, selection by farmers and, more recently, efforts of plant breeders have each played a role. Many successful varieties in any ecological, agricultural, or societal situation have survived for long periods despite the release of numerous "improved" varieties intended as replacements. Enduring varieties and inbred lines are obviously the ones to be considered seriously for choice as recurrent parents in backcross breeding programs.

### *Maintenance of the Character Under Transfer*

In backcross breeding the level of heritability is rarely a problem of any consequence except for the character(s) under transfer, but for such characters high heritability is important. This is because selection must be practiced for the character(s) being transferred through each of several generations of backcrossing. At the same time all other characters will be taken care of automatically by the backcrossing procedure, with the result that the breeder need pay them little or no attention. Backcross breeding thus has its greatest ease of application when the character being transferred can be readily identified by visual inspection or by other quick and simple tests. As with all methods of breeding, characters governed by single alleles are easiest to manage. However, backcross breeding is by no means restricted to characters governed by alleles of one or even several genes. The number of alleles involved is often less important than the precision with which the character under transfer can be identified in segregating populations; a character of high heritability governed by several alleles might well be more easily transferred than a character of low heritability governed by only one allele. Regardless of the number of alleles governing the character to be transferred, it is essential that a worthwhile intensity of the character be maintained throughout a series of backcrosses. Some of the intensity of a character may be lost even when the control is primarily monogenic. This is often true in the transfer of disease-resistance alleles. Backcross-derived disease-resistance varieties carrying a "single" resistance allele may show some symptoms of disease when artificially inoculated, but may show no symptoms or, at the least, escape measurable damage under agricultural conditions. In some cases, even though there are some symptoms of disease, the causal organism has often been unable to maintain itself and the disease ceases to be a significant factor. Sooner or later, however, most pathogenic organisms "fight back" by developing novel virulent races. It has been possible in some cases to anticipate and circumvent this contingency by backcrossing additional potent resistance alleles into commercial varieties and deploying them if and when they are needed. This stratagem has proved effective in combating scald disease, caused by *Rhynchosporium secalis*, a highly resourceful foliar disease of barley characterized by numerous races governed by alleles of many different loci.

In dealing with quantitative characters, the donor parent may be chosen with the idea of sacrificing some of the intensity of the character to be transferred. Thus, an adapted short-grain variety of rice has sometimes been chosen as the recurrent

parent and a long-grain parent as the donor parent in breeding medium-grain varieties of rice with the adaptedness and yielding ability of short-grain varieties.

### *The Number of Backcrosses*

If backcross breeding programs are to succeed with certainty, the complex, essential features of the genotype of the recurrent parent that endow it with superior overall worth must be recovered. Such recovery will almost certainly be primarily a function of the number of backcrosses, even though selection for the general type of the recurrent parent, especially in early backcross generations, can be effective in rapidly shifting the population toward the characteristics of that parent. Furthermore, the recurrent parent is frequently not composed of a single pure line but of a number of closely related pure lines that interact synergistically to give the recurrent parent its agricultural characteristics. Hence, enough plants of the recurrent parent must be involved in the backcrossing program to recapture the overall agricultural worth of the recurrent parent. Six backcrosses coupled with careful selection for the type of the recurrent parent, especially in early generations, has become more or less standard in backcross breeding programs in selfing species. It is commonly accepted that selection for the type of the recurrent parent, if based on moderate-size populations, is equivalent in effectiveness to one or two additional backcrosses without selection for type. After the third backcross, however, the population is likely to resemble the recurrent parent so closely that human-directed selection, on an individual plant basis, is largely ineffective except for intensity of the character being transferred.

Backcrosses can also serve a useful purpose in pedigree and bulk-population breeding programs. When, in a pedigree or bulk-population program, one of the parents is superior to the other parent in most of its characteristics, and especially if it is superior in overall worth, it is often advantageous to backcross the  $F_1$  hybrid to the superior parent. One backcross will skew the segregating generations sharply toward that parent, thereby likely increasing the frequency of superior genotypes in late-segregating generations. Another alternative is to skew the segregation even more toward the superior parent by crossing the first backcross generation to the superior parent. Stopping the backcrossing early preserves the likelihood that transgressive segregation will contribute more than just a single superior desirable allele to the recurrent parent. However, a price attends this procedure, namely, relinquishment of control over the segregation that the backcross method regulates so precisely. As a consequence, the evaluation trials that are not necessary in standard backcross programs are necessary with programs that employ just "some" backcrossing. Programs that feature only "some" backcrossing are perhaps more accurately described as "backcross pedigree" or "backcross bulk" programs rather than as true backcross programs.

### *Backcross Breeding Procedures*

The general plan by which many backcross breeding programs have been carried out is as follows:

1. Selection of parents: The recurrent parent (parent A) is almost always a paramount variety of the area or an elite inbred line in outcrossing species, whereas the donor parent (B) usually possesses in high intensity some desirable, usually simply inherited, attribute in which parent A is deficient.

2. The  $F_1$  of the hybrid of  $A \times B$  is backcrossed to parent A, giving a progeny segregating for the simply inherited desirable character of parent B. Several individuals with the desirable character of parent B are selected and allowed to self-pollinate to produce  $B_1F_2$  families, among and within which intensive selection is practiced for high intensity of the desirable character of parent B and for the general features of the recurrent parent (parent A). The  $B_1F_2$  plants are allowed to self-pollinate to produce  $B_1F_3$  lines, among and within which further intense selection is practiced for high expression of the desirable trait of parent B and for the general type of the recurrent parent (parent A).

3. The selected  $B_1F_3$  plants are again backcrossed to parent A to produce  $B_2F_2$  ( $A^3 \times B$ ) seeds. Plants from these seeds are backcrossed yet again to parent A to produce third backcross ( $A^4 \times B$ ) seeds.

4. The procedure of step 2 is repeated, with the fourth, fifth, and sixth backcrosses made in succession. An  $F_2$  and  $F_3$  are usually grown only after the sixth backcross, at which time intensive selection is practiced for the character being transferred, as well as for the general type of the recurrent parent.

5. A number of lines homozygous for the character being transferred, which are also as similar to the recurrent parent as possible, are selected, bulked, and the bulk increased and released for commercial production.

If appropriate numbers of plants are grown at each step in the foregoing program, the transfer of a single dominant allele can usually be accomplished through five backcrosses ( $A^6 \times B$ ) with 53 plants from backcrossed seeds, 96  $F_2$  plants and 68  $F_3$  rows of 24 plants each. These numbers provide 0.999 probability of having at least one  $Aa$  plant after each backcross and at least one homozygous  $AA$  progeny in each  $F_3$ . In the case of species in which hand-pollinated artificial hybridizations are difficult (e.g., small-flowered legumes), the number of hybrid seeds required can be reduced by growing selfed  $F_2$  and  $F_3$  populations after each backcross and crossing on homozygotes. Somewhat smaller total populations are required to transfer incompletely dominant or recessive alleles, provided homozygotes can be recognized, making  $F_3$  progenies unnecessary. Note that the program outlined previously does not call for successive backcrosses but for growing  $F_2$  and  $F_3$  generations only after the first, third, and sixth backcrosses. Note also that substantially larger numbers of plants are sometimes called for than the theoretical minima. The reasons for this are grounded in two considerations believed to outweigh the advantages of successive backcrossing and theoretically minimal population sizes. First, there is compelling evidence that selection for the types of the recurrent parent in  $F_2$  or  $F_3$  populations of modest size is equivalent to at least one, and perhaps even two, additional backcrosses in a continuous series without rigorous selection. Second, selfing after the first, third, and sixth backcrosses requires little effort and provides the breeder with populations sufficiently large to permit selection to be practiced for worthwhile intensity of expression of the character under transfer, as well as for the type of the recurrent parent.

*Improvement by Steps*

Although the backcross method has its most straightforward application in the transfer of single monogenic characters, it also offers a number of procedures by which a variety can be improved for two or more monogenic characters more or less simultaneously, or by which additional alleles affecting one character can, if needed, be accumulated in one variety. Once a variety has been improved in one or more characteristics, the use of the latest backcross-derived individuals as recurrent parents will, of course, automatically preserve all previous improvements. This has frequently been the case in breeding for disease resistance in which resistance to some particularly damaging disease was first incorporated into a dominant variety, followed by later incorporation of resistance to less damaging disease using the earlier resistant genotypes as recurrent parents. In some cases the appearance of new disease races has made it necessary to add additional protective alleles. In other cases awns or other morphological characteristics were added because they had been found to improve yields and/or quality of product.

Another approach that has been used to improve a variety in two or more characteristics is to transfer the necessary alleles simultaneously in the same program. This procedure has the disadvantage that somewhat larger populations are required to transfer two alleles simultaneously than to transfer them separately. This disadvantage is even greater with three or more independent loci, because genetic complexity increases exponentially with the number of loci. Further, some characters may not be expressed dependably, thus delaying both or all transfers.

*Transferring Quantitative Characters*

Backcross breeding is by no means restricted to transfers of traits governed by major genes. It has frequently been used to adjust the expression of quantitatively inherited characters, such as early flowering. In this case the usual procedure has been to cross a dominant variety or popular inbred line (the recurrent parent) with an early flowering stock (the donor parent), backcross to the recurrent parent, select and self-pollinate a number of  $BC_1$  early plants that most closely resemble the recurrent parent, then select for earliness and for the plant type of the recurrent parent in the selfed  $BC_1F_2$  family lines. Repeating this cycle one or more times will often provide early lines that adequately resemble the recurrent parent. In many cases it has been found that one or a very few alleles have major effects on earliness (and on other quantitative characters) and that the aforementioned procedure identifies these alleles and fixes them in individuals that are otherwise sufficiently like the recurrent parents. Thus important adjustments can often be made in quantitative characters without undue sacrifice in the essential properties of the recurrent parent.

*Applications to Cross-Pollinated Crops*

Backcross improvement of outbreeding species differs in no fundamental way from that of self-pollinated species. However, with outcrossing species particular care is required to ensure that the sample of gametes taken from the recurrent parent

accurately represents the allelic frequencies of that parent. The breeding of Caliverde alfalfa is a good example of the application of backcrossing to an outcrossing species. Mildew-resistant and leaf-spot-resistant plants found in California Common, the recurrent parent of Caliverde, were the source of resistance to these two diseases. Resistance of alfalfa to wilt disease was transferred from Turke-san, a winter-hardy type wholly unsuited for commercial production in California. About 200 plants of California Common were used to represent the recurrent parent in each of four backcrosses of resistant plants to the recurrent parent. The breeding of this variety, including seed increase for release to commercial growers, was completed in 7 years. Caliverde, aside from its resistance to mildew, leaf spot, and bacterial wilt diseases, was indistinguishable from California Common; it rapidly and completely replaced its recurrent parent in the commercial production of alfalfa in California.

### *Influence of Environmental Conditions on Backcross Programs*

One of the advantages of the backcross method, especially if the recurrent parent is a homozygous inbreeder, is that the program can be carried out in almost any environment that allows development of the character under transfer. With pedigree or bulk-population breeding programs the use of greenhouses or off-season nurseries is usually limited to tests for disease resistance or to adding generations, because efforts to evaluate agricultural performance are, in large part, wasted except under conditions closely approximating normal growing conditions of the resulting variety. Backcross programs are, for the most part, not subject to this handicap because adequate backcrossing automatically restores the genotype of the recurrent parent. Hence, backcross programs can often be accelerated by growing additional generations in off-season nurseries, in greenhouses, or in cold frames. However, caution must be exercised if the recurrent parent is highly variable genetically and subject to rapid modification by selection, as may be the case with many varieties maintained by free outbreeding, such as alfalfa.

### USAGE OF BACKCROSSING IN PLANT BREEDING

Most of the examples discussed in this chapter have featured breeding for disease resistance. This is appropriate, because the backcross method has been used for this purpose perhaps more than for any other single purpose. Moreover, many of the early descriptions of backcross breeding in the plant breeding literature were based on transfers of dominant monogenic resistance alleles into highly homozygous cereal varieties. It should be emphasized, nevertheless, that the backcross method is suited for numerous other purposes as well, including transfers of recessive alleles and adjustment of any trait under transfer that is at least moderately heritable. Although the early literature is replete with successful applications of the method, more recent applications have usually not been publicized. One reason is that the method is so simple and straightforward that many breeders have regarded announcement of results as superfluous. Another, unjustified, reason is that some

breeders have thought that publicizing the results of such a simple, straightforward, and predictable method might expose them to being labeled "hack" breeders.

The main reason the backcross method has come to be particularly associated with breeding for disease resistance is probably related to the devastating effects that rusts can have on the productivity and quality of wheat, the "staff of life." The place of North American hard-red-winter and hard-red-spring wheats in world markets depends on their abundance and noted baking quality. The appearance of new and devastating races of rust that occurred with disturbing regularity in the 1940s was a matter of international concern, especially the appearance of the exceptionally virulent race, 15B, of stem rust. New high-yielding varieties of wheat resistant to these races had to be developed quickly, and programs of backcross breeding that avoided the necessity of lengthy evaluation trials to recover important quality characteristics were widely adopted for this purpose. The danger of losses owing to new physiological races of rust was undoubtedly the main factor that induced many North American wheat breeders to adopt backcross programs, which succeeded spectacularly. The method was also used widely in South America and Australia. In Europe, Asia, and Africa, however, where cereal diseases are generally less destructive, the backcross method has been used considerably less frequently. Nevertheless, the value of the backcross method for handling a wide variety of problems in plant breeding has been amply demonstrated, and the use of the method has continued, but nearly always with little fanfare.

Another aspect of backcross breeding has been described by Borlaug (1957) as follows:

There are many undesirable aspects associated with precipitous changes in varieties. Farmers are reluctant to shift from a proven variety to a new unknown variety. Their reluctance is based on familiarity with the old variety which permits them to exploit to a maximum its potential yielding ability. They know the best rates and dates of seeding of the old variety for their local conditions. When a new variety is introduced these considerations must again be worked out by the grower for his own local conditions before he is able to utilize the new variety in such a way as to approach its potential optimum productivity. . . . The conventional backcross method of plant breeding comes closest to overcoming this problem. It provides, if properly carried out, new varieties which are for all practical purposes phenotypically identical to the recurrent parent and therefore readily received by farmers, processors and consumers.

Many breeders have not followed the conventional backcross process to reconstitute the recurrent parent precisely, but have used only one, two, or three backcrosses to retain the possibility of segregation for agronomic or horticultural characteristics such as yield and adaptedness. The possible benefits of compromises of this sort are enticing, but there are serious possible disadvantages. Time has been and remains a particularly important factor in the production of new varieties; it usually takes as long or longer to evaluate properly the potential of a new variety than it takes to produce and to bring such a variety to the point at which intensive evaluation can begin. Moreover, evaluation in one place can rarely be assumed to

be accurate except, possibly, for that particular place. As a result of the pressures of time and expense, many "promising" genotypes have been released to commercial production prematurely. In addition, the success or attainment of eminence of breeders, and the institutions they represent, has sometimes been measured by the total numbers of varieties that breeders have released. This is certainly a much less appropriate measure of accomplishment and achievement than the number and the extent of success of the truly worthy varieties that breeders have released to commercial production. The success of the backcross method has been amply demonstrated in situations of emergency. In such situations it appears both reasonable and prudent for breeders to put first things first, that is, to confront any potential emergency directly and without delay, relegating less pressing matters to secondary positions of priority. This leaves open the question as to when less certain breeding methods that depend on transgressive segregation should be instituted. An issue such as this clearly depends on how long it is likely to take to develop a variety capable of superseding a backcross-derived emergency variety in overall worth. It is possible, but unlikely, that this might happen soon enough that a backcross-derived emergency variety need never be released; however, should this happen, little wastage will likely have occurred because backcross programs require minimal expenditures of time, labor, and other resources—and even inexperienced breeders rarely fail to produce a worthwhile product.



## Education Center

### Related Links

- 1 The Process of Plant Genetic Engineering
- 2 DNA and DNA Extraction
- 3 Gene Cloning
- 4 Gene Regions
- 5 Transformation
- 6 Backcross breeding

## Overview of the Process of Plant Genetic Engineering

The improvement of crops with the use of genetics has been occurring for years. Traditionally, crop improvement was accomplished by selecting the best looking plants/seeds and saving them to plant for the next year's crop.

Once the science of genetics became better understood, plant breeders used what they knew about the genes of a plant to select for specific desirable traits. This type of genetic modification, called traditional plant breeding, modifies the genetic composition of plants by making crosses and selecting new superior genotype combinations. Traditional plant breeding has been going on for hundreds of years and is still commonly used today.

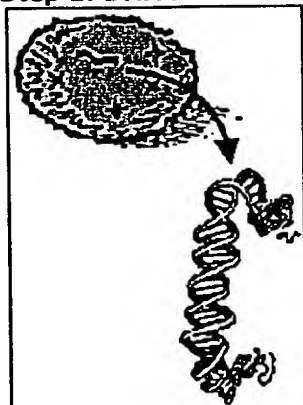
Plant breeding is an important tool, but has limitations. First, breeding can only be done between two plants that can sexually mate with each other. This limits the new traits that can be added to those that already exist in that species. Second, when plants are mated, (crossed), many traits are transferred along with the trait of interest including traits with undesirable effects on yield potential.

Genetic engineering is a new type of genetic modification. It is the purposeful addition of a foreign gene or genes to the genome of an organism. A gene holds information that will give the organism a trait. Genetic engineering is not bound by the limitations of traditional plant breeding. Genetic engineering physically removes the DNA from one organism and transfers the gene(s) for one or a few traits into another. Since crossing is not necessary, the 'sexual' barrier between species is overcome. Therefore, traits from any living organism can be transferred into a plant. This method is also more specific in that a single trait can be added to a plant.



Traits, such as seed color, are controlled by DNA.

### Step 1: DNA Extraction



Extracting DNA from an organism.

The process of genetic engineering requires the successful completion of a series of five steps.

DNA extraction is the first step in the genetic engineering process. In order to work with DNA, scientists must extract it from the desired organism. A sample of an organism containing the gene of interest is taken through a series of steps to remove the DNA.

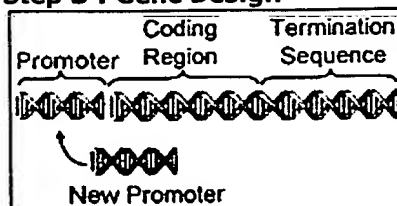
### Step 2 : Gene Cloning

The second step of the genetic engineering process is gene



cloning. During DNA extraction, all of the DNA from the organism is extracted at once. Scientists use gene cloning to separate the single gene of interest from the rest of the genes extracted and make thousands of copies of it.

### Step 3 : Gene Design



Once a gene has been cloned, genetic engineers begin the third step, designing the gene to work once inside a different organism. This is done in a test tube by cutting the gene apart with enzymes and replacing gene regions that have been separated.

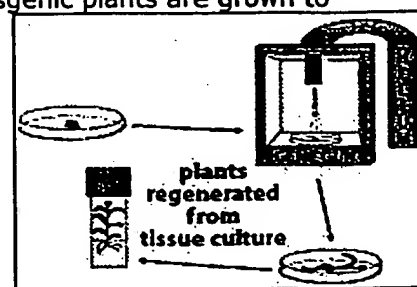
**Replacing an existing promoter with a new promoter.**

### Step 4 : Transformation

The modified gene is now ready for the fourth step in the process, transformation or gene insertion.

Since plants have millions of cells, it would be impossible to insert a copy of the transgene into every cell. Therefore, tissue culture is used to propagate masses of undifferentiated plant cells called callus. These are the cells to which the new transgene will be added.

The new gene is inserted into some of the cells using various techniques. Some of the more common methods include the gene gun, agrobacterium, microfibers, and electroporation. The main goal of each of these methods is to transport the new gene (s) and deliver them into the nucleus of a cell without killing it. Transformed plant cells are then regenerated into transgenic plants. The transgenic plants are grown to maturity in greenhouses and the seed they produce, which has inherited the transgene, is collected. The genetic engineer's job is now complete. He/she will hand the transgenic seeds over to a plant breeder who is responsible for the final step.



**Using the gene gun method to transform plant cells.**

### Step 5 : Backcross Breeding



The fifth and final part of producing a genetically engineered crop is backcross breeding. Transgenic plants are crossed with elite breeding lines using traditional plant breeding methods to combine the desired traits of elite parents and the transgene into a single line. The offspring are repeatedly crossed back to the elite line to obtain a high yielding transgenic line. The result will be a plant with a yield potential

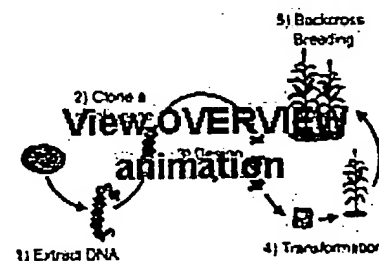
close to current hybrids that expresses the trait encoded by the new transgene.

### The Process of Plant Genetic Engineering

The entire genetic engineering process is basically the same for any plant. The length of time required to complete all five steps from start to finish varies depending upon the gene, crop species, available resources and regulatory approval. It can take anywhere from 6-15+ years before a new transgenic hybrid is ready for release to be grown in production fields.

Take a self-study quiz on the process of genetic engineering.

Want to learn more about the process of genetic engineering?





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### Backcross Breeding

Some crop lines are genetically more equipped to handle the stresses of tissue culture. Since these lines are typically lower yielding, older lines, once a plant is successfully transformed it must go through backcrossing to move the transgene into a high yielding, elite line.

Gene cloning is the process in which a gene of interest is located and copied (cloned) out of all the DNA extracted from an organism. Since there is no way to locate a gene by visibly looking at all of the DNA, scientists make gene libraries to catalogue the organism's DNA and then select the gene he/she is looking for from this library.



Plant breeding is the final step of making a marketable transgenic line

Why is it necessary to take the time and effort to do backcrossing? Backcrossing is necessary because the lines that lend themselves well to tissue culture and transformation are typically older, low yielding lines. Plant breeders use backcrossing to transfer the transgene from these older lines into elite, high yielding lines. Also, plant breeders can screen for the presence of negative mutations that occurred during the transformation process.

### How is backcross breeding done?

The backcross breeding method has been used by plant breeders for decades to incorporate specific traits into elite lines. This method works by crossing the transgenic inbred line with an elite inbred line of choice. In the following step, the breeder crosses the selected transgenic offspring back to the elite inbred again. This process of crossing back to the elite line (backcrossing) is repeated until the offspring has 99+% elite genes and the transgene. plant breeding method.

### Identifying Transgenic Plants

How do breeders determine which plants express the trait encoded by the transgene?

One option is for the breeders to observe the plant for the trait of interest. However, if the trait is not easily detectable in the field (such as seed protein) then this method is inefficient.

Another option is to utilize the selectable marker that was inserted during transformation. However, this is not feasible if the selectable marker gene was an antibiotic resistance gene. Also, the selectable marker gene sometimes inserts into a different chromosome than the transgene. As a result, some offspring will have the transgene and not the selectable marker gene and vice versa.

Plant breeders often use an ELISA test, which detects the presence of the protein encoded by the transgene in a sample of plant tissue. This test can be quickly done in the field using a kit and a small sample of tissue and will give a simple positive or negative response.

Sometimes a more sophisticated test called PCR is used. The second method tests for the presence of the transgene itself. This laboratory method, called PCR (Polymerase Chain Reaction), is much more time consuming and expensive. However, it can be used to detect the presence of a transgene in tissues that are not expressing the gene.

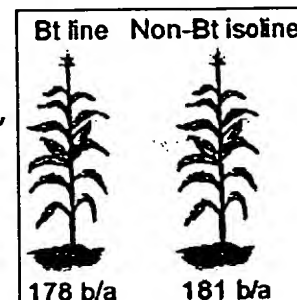
A final option sometimes used by major companies is genetic fingerprinting. This technique can be used to not only identify the presence of the transgene, but find which plants, through the natural variation in breeding, have obtained a greater percentage of the elite inbred genes. This can potentially shorten the number of generations required for backcrossing. Although very advantageous, this technique is also very time consuming and expensive, and could not be performed on a large number of lines.

All of these detection methods are important tools that help the plant breeder identify the plants they should be making crosses with.

It takes the breeder at least two or three years to derive a backcross line that is the genetic equivalent of the elite line plus the event. After that point the plant breeder can work with the genetically engineered line in the same manner they work with other parents in their breeding program. Many companies have taken advantage of genetic fingerprinting technology and year-round nurseries to maximize the efficiency and speed of backcross line development.

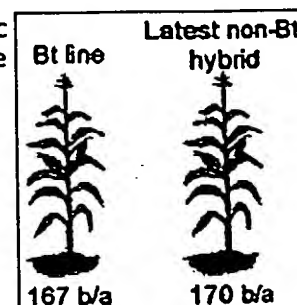
### Yield Drag and Yield Lag

Yield drag is the negative effect on yield potential associated with crop plants that have a specific gene or trait. Yield drag can be caused by the transgene may inserting into a gene important for plant growth and yield disrupting its expression, or it can be caused by a drain in the limited pool of amino acids due to having to produce large quantities of new proteins. This limits the amount of amino acids available for the production of other proteins important to plant growth and yield. Genetically engineered crops are not necessarily more prone to yield drag or yield lag than non-genetically engineered crops. However, genetically engineered crops are unique in that an additional gene is being placed into the chromosome.



Yield drag is apparent when a transgenic line yields less than its isolate

Yield lag is a difference in yield potential between a transgenic line and the newest elite lines. The difference is because there is no selection for increased yield during the 3-5 years of backcross breeding while non-transgenic lines have had selection for improved yield potential every year. Therefore the lines coming out of a backcrossing program have gone through a "lag period" in which the plant breeder has not imposed selection for yield. These lines would be expected to experience a yield lag.



Yield lag is apparent when a transgenic line yields less than a newly released elite line.

Once a transgene has been backcrossed into an elite inbred background, it is no longer necessary for it to undergo backcrossing again. It can be used in the same manner as non-transgenic lines in breeding programs being mated to other lines and undergoing selection for improved qualities including yield. Thus, over time yield lag is no longer an issue in a particular transgenic event.

### Gene Stacking

Gene stacking is a term that is used in the context of genetically engineered crops, but is not a new idea in plant breeding. Gene stacking is combining desired traits into one

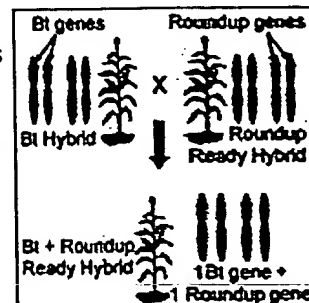
line. Plant breeders are always stacking genes by making crosses between parents that each have a desired trait and then identifying offspring that have both of these desired traits. This is the quickest and easiest way to stack genes. Another way to stack genes is by transferring two or more genes into the cell nucleus during transformation. The use of a selectable marker in addition to the gene of interest would be considered gene stacking.

The effect of the transgenes on the overall metabolism of the plant will probably be the major limitation to how many genes and what combinations of genes we can stack into a crop plant. Right now we are only asking the plant to make a few copies of one or two additional proteins. If the transgenes are designed to change protein synthesis and metabolism more dramatically, it is likely that the productivity of the plant will be compromised and yield drag will result. The loss of yield will need to be offset by cost savings in production or extra value for the grain.

Take a self-study quiz on this topic

Want to learn more about gene inheritance?

Want to learn more about backcross breeding?



Mating a homozygous Roundup resistant plant with a homozygous Bt plant will result in progeny stacked for both traits. The progeny will have one copy of the Roundup resistance gene and the Bt gene.